Toxicology Letters 182 (2008) 50–56

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

# Transplacental transfer of acrylamide and glycidamide are comparable to that of antipyrine in perfused human placenta

Kirsi Annola[a], Vesa Karttunen[a], Pekka Keski-Rahkonen[b], Päivi Myllynen[c], Dan Segerbäck[d], Seppo Heinonen[e], Kirsi Vähäkangas[a,*]

[a] Department of Pharmacology and Toxicology, University of Kuopio, P.O. Box 1627, FIN-70211 Kuopio, Finland
[b] Department of Pharmaceutical Chemistry, University of Kuopio, P.O. Box 1627, FIN-70211 Kuopio, Finland
[c] Department of Pharmacology and Toxicology, P.O. Box 5000, University of Oulu, FIN-90014 Oulu, Finland
[d] Department of Biosciences and Nutrition, Karolinska Institute, Novum, S-141 57 Huddinge, Sweden
[e] Department of Gynaecology and Obstetrics, Kuopio University Hospital, P.O. Box 1777, 70211 Kuopio, Finland

ARTICLE INFO

*Article history:*
Received 11 February 2008
Received in revised form 10 June 2008
Accepted 13 August 2008
Available online 23 August 2008

*Keywords:*
Fetal exposure
DNA adducts
Xenobiotic metabolism
Food carcinogens

ABSTRACT

Most drugs can penetrate the placenta but there are only a few studies on placental transfer of environmental toxic compounds. In this study, we used dual recirculating human placental perfusion to determine the transfer rate through the placenta of a neurotoxic and carcinogenic compound found in food, acrylamide and its genotoxic metabolite glycidamide. Putative acrylamide metabolism into glycidamide during the 4-h perfusions and acrylamide-derived DNA adducts in placental DNA after perfusions were also analyzed. Placentas were collected immediately after delivery and kept physiologically functional as confirmed by antipyrine kinetics, glucose consumption and leak from fetal to maternal circulation. Acrylamide (5 or 10 μg/ml) or glycidamide (5 μg/ml), both with antipyrine (100 μg/ml), was added to maternal circulation. Acrylamide and glycidamide were analyzed in the perfusion medium by liquid chromatography/mass spectrometry. Acrylamide and glycidamide crossed the placenta from maternal to fetal circulation with similar kinetics to antipyrine, suggesting fetal exposure if the mother is exposed. The concentrations in maternal and fetal circulations equilibrated within 2 h for both studied compounds and with both concentrations. Acrylamide metabolism into glycidamide was not detected during the 4-h perfusions. Moreover, DNA adducts were undetectable in the placentas after perfusions. However, fetuses may be exposed to glycidamide after maternal metabolism. Although not found in placental tissue after 4 h of perfusion, it is possible that glycidamide adducts are formed in fetal DNA.



## 1. Introduction

Acrylamide (AA) has been one of the most investigated toxic compounds among food carcinogens during recent years. In 2002 it was found in high concentrations in various food products, especially in potato chips (Tareke et al., 2002). It has become evident that human exposure to AA is mainly via food, where it is formed when heating food rich in carbohydrates over 180 °C (Wirfält et al., 2007). However, the exposure via tobacco smoke is even higher. The typical average daily intake to AA may vary from 0.3 to 2 μg/kg bw/day. However, the intake can reach even 5 μg/kg bw/day (Parzefall, 2008). In animals AA is neurotoxic, mutagenic and carcinogenic, and disturbs fetal development and reproductive functions (Tyl and Friedman, 2003). Fetotoxic effects of AA include neurotoxicity (Dearfield et al., 1988) and other effects such as reduced fetal body weight, decreased number of offspring and infertility (Field et al., 1990; Sakamoto and Hashimoto, 1986; Tyl and Friedman, 2003; Zenick et al., 1986). While also neurotoxic in humans, epidemiologic studies on AA have so far not indicated any carcinogenic effects (Exon, 2006). However, on the basis of animal studies and genotoxicity, AA is classified by IARC (1994) into group 2A: probably carcinogenic to humans. According to Besaratinia and Pfeifer (2004) AA induces mainly mutations through metabolism to glycidamide (GA). While GA is clearly, but moderately mutagenic (Baum et al., 2005), the genotoxicity of AA is different: it has been shown to be mainly clastogenic in human cells (Koyama et al., 2006). GA can also induce apoptosis and inhibit DNA repair (Blasiak et al., 2004; Dearfield et al., 1988). GA reacts with DNA forming DNA adducts mainly with N7-guanine and N1 and N3 of adenine. Lesions have

*Abbreviations:* AA, acrylamide; GA, glycidamide; LC/MS, liquid chromatography/mass spectrometry.

* Corresponding author at: Department of Pharmacology and Toxicology, University of Kuopio, P.O. Box 1627, FIN-70211 Kuopio, Finland. Tel.: +358 40 745 5254.
*E-mail address:* kirsi.vahakangas@uku.fi (K. Vähäkangas).

0378-4274/$ – see front matter © 2008 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.toxlet.2008.08.006

been detected in rodents exposed to AA or GA (Segerbäck et al., 1995; Gamboa da Costa et al., 2003; Doerge et al., 2005).

During the last decades there has been increasing interest in developmental toxicology with growing awareness that a comprehensive risk assessment should also involve the assessment of fetal exposure and effects (Anderson et al., 2000). For fetal risk assessment it is important to learn more about the transplacental transfer of toxic compounds in human placenta. Studies with human placenta are crucial because of the functional differences in anatomy and physiology of the placenta between different species (Faber, 1995; Leiser and Kaufmann, 1994). Evidence from animals and humans shows that the *in utero* exposure can induce toxic effects including cancer later in life (Anderson et al., 2000; Miller, 2004). While only one proven transplacental chemical carcinogen is known in humans, diethylstilbestrol, many more are suspected (Anderson et al., 2000). An *ex vivo* human placental perfusion has appeared useful for the analysis of transplacental transfer of drugs (Ala-Kokko et al., 2000; Vähäkangas and Myllynen, 2006). It has also been used in studies of metals and minerals, e.g. mercury, cadmium and selenium (Eisenmann and Miller, 1994; Urbach et al., 1992), ethanol (Karl et al., 1988), nicotine (Pastrakuljic et al., 1998) and phthalates (Mose et al., 2007). Furthermore, placental perfusion may give information of toxic responses in placental tissue induced by the perfused compounds. Because a well-functioning placenta is a prerequisite for fetal development, any disturbance in its functions by toxic compounds like environmental carcinogens may affect the fetus as well.

We have studied transplacental transfer of AA and its active metabolite, GA in human placental perfusion. There is only one study published on the transfer of AA through human placenta with three short-term placental perfusions (Sörgel et al., 2002). Because the placenta is capable of xenobiotic metabolism (Vähäkangas and Myllynen, 2006), and GA can bind to DNA (Segerbäck et al., 1995; Xie et al., 2006), we also analyzed AA metabolism and AA and GA DNA adducts in the perfused placenta.

## 2. Methods

### 2.1. Reagents

AA (99.9%) and antipyrine were purchased from Sigma (St Louis, Mo, USA), GA from Toronto Research Chemicals (Toronto, Canada) and $^{13}$C-AA (1,2,3-$^{13}$C3, 99%, 1 mg/ml in methanol) from Cambridge Isotope Laboratories (Andover, MA, USA). All reagents were of analytical grade, and the solvents were of HPLC grade.

### 2.2. Human placentas

In Finland, human placenta is disposable after delivery and the use of the placenta does not affect the delivery or the treatment of the mother and child in any way. The official Research Ethics Committee of the University Hospital District of Kuopio region approved the study protocol (11.5.2005). Only full-term placentas from healthy non-smoking mothers were used in the study. Mothers were not under medical treatment and they stated that they did not use alcohol during pregnancy. Mothers were informed about the study by the midwife and invited to participate. Those who agreed signed an informed consent with written information. The placentas were anonymized. Altogether 32 placentas were obtained after a normal delivery or caesarean section.

### 2.3. Human placental perfusion

#### 2.3.1. Perfusion equipment, method and samples

Maternal and fetal circulations were perfused separately using a dual recirculating perfusion method described earlier (Pienimäki et al., 1995; Myllynen et al., 2003) (Fig. 1). The placentas were obtained within 10 min after the delivery and examined for macroscopic traumas and hematomas. Fifteen millilitres of heparinised (25 IU/ml, Leo-Pharma) Krebs–Ringer buffer solution was injected into all cord vessels to prevent blood coagulation in the placenta. A peripheral lobule was cannulated to establish the fetal circulation (approximately 1 ml/min). If venous outflow corresponded to inflow, the perfused placental lobule was cut out and transferred into a custom-made chamber and the chamber with the lobule maternal side upwards was placed into the perfusion equipment. In the maternal side cannules were pushed through the membrane to the maternal blood space. The first 50 ml of maternal perfusate was discarded to remove red blood cells before connecting the recirculating maternal flow.

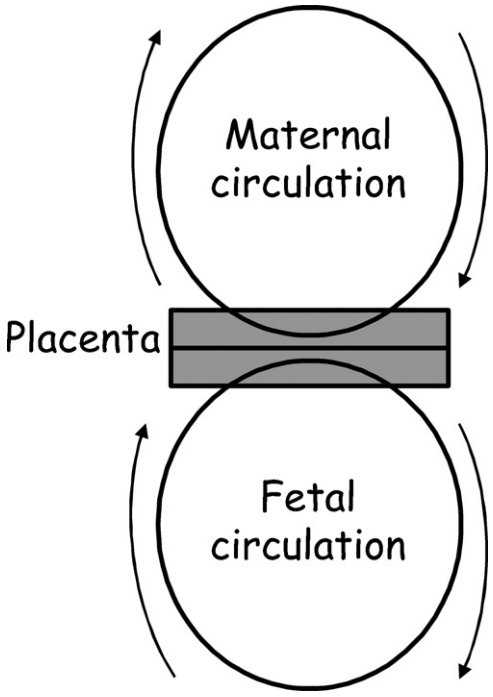


**Fig. 1.** Principle of the dual, recirculating placental perfusion, where the placental structure and the barrier between fetal and maternal circulations remain intact, mimicking thus the physiological *in vivo* conditions.

Perfusion medium used was RPMI 1640 cell culture medium (Cambrex, Verviers, Belgium) with dextran (2 g/l, Sigma), albumin (2 g/l, SPR, Finland), heparin (25 IU/ml, Leo Pharma, Malmö, Sweden), sodium pyruvate (1 mM, Cambrex), non-essential amino acid-solution (10 ml/l, Cambrex), Penicillin–Streptomycin (25 U/ml, Cambrex) and L-glutamine (2 mM, Cambrex) in both circulations. The final volume in maternal circulation was 200 ml and in fetal compartment 120 ml. Perfusion medium was gassed with nitrogen/oxygen (95%/5%) in the fetal side and oxygen/carbon dioxide (95%/5%) in the maternal side. The flow rate of the perfusate was 3 ml/min in the fetal and 9 ml/min in the maternal side. Thirty to 45 min was given for the placenta to recover from hypoxia and control samples were drawn from circulation at the end of the recovery period. Thereafter, AA (5 or 10 μg/ml) or GA (5 μg/ml) was added into the maternal circulation, both with antipyrine (0.1 mg/ml). Antipyrine, that crosses placenta via passive diffusion (Brandes et al., 1983; Challier, 1985; Rurak et al., 1991; Schenker et al., 1992), was used as a reference substance in all perfusions. The physicochemical properties of AA, GA and antipyrine are shown in Table 1. After addition of the study compounds, perfusions were carried out mainly for 4 h and one perfusion for 6 h. Samples (1.6 ml) from both circulations were taken every 30 min during the first 2 h and once an hour thereafter. Perfusion fluid samples were centrifuged at 12,000 × *g* for 15 min to remove red blood cells and stored in freezer (−20 °C) until analyzed. For DNA adduct analysis, pieces of the perfused tissue were snap-frozen in liquid nitrogen and stored in −80 °C. Control samples from the tissue were taken before the perfusion. Altogether nine successful AA perfusions were carried out with 10 μg/ml and four with 5 μg/ml. With GA 5 μg/ml, four successful perfusions were performed.

#### 2.3.2. Criteria for a successful perfusion

The main criterion for a successful perfusion was the absence of leak from the fetal to maternal circulation (Pienimäki et al., 1995; Table 2). The volume of the fetal perfusate was monitored continuously to determine the absence of leak from the fetal circulation as an indication of the integrity of the materno-fetal barrier. If the volume loss exceeded 3 ml/h, the experiment was terminated. Accordingly, if the expected antipyrine transfer from maternal to fetal circulation was less than optimal (fetal to maternal concentration ratio below 0.33 at 4 h (Challier et al., 1983) the perfusion was omitted from data analysis. Antipyrine is used in perfusions for comparison, to see whether the fetal and maternal circulations overlap properly and to normalize the putative interindividual variation in circulation (Ala-Kokko et al., 2000; Ching et al., 1987; Ghabrial et al., 1991; Myllynen et al., 2003; Pienimäki et al., 1995, 1997). Oxygen, carbon dioxide and pH were monitored once an hour using a blood gas analyser (Stat Profile pHOx Basic). pH was adjusted with 1 M HCl if outside the accepted range (7.4 ± 0.1). The glucose consumption of placental tissue was ana-